Amendments to the Claims

Claim 1 (currently amended): A separation matrix ~~comprised of~~ comprising a porous support; and ligands coupled to the surfaces of ~~a~~ said porous support, wherein the ligands provide at least one chemical gradient in the support.

Claim 2 (currently amended): ~~A~~ The separation matrix ~~according to~~ of claim 1, wherein the support comprises porous particles and the ligand gradient(s) extend between the ~~centre~~ center and the exterior surface of each porous particle.

Claim 3 (currently amended): ~~A matrix according to claim 1 or 2,~~ The separation matrix of claim 1, wherein at least one gradient is a ligand density gradient formed by a changing density of ligands on the support.

Claim 4 (currently amended): ~~A~~ The separation matrix ~~according to~~ of claim 3, wherein two or more chemical gradients are present in the support and at least one gradient is a ligand density gradient.

Claim 5 (currently amended): ~~A matrix according to claim 3 or 4,~~ The separation matrix of claim 3, wherein in the ligand density gradient(s), the ligand concentration increases towards the ~~centre~~ center of the support.

Claim 6 (currently amended): ~~A matrix according to claim 3 or 4,~~ The separation matrix of claim 3, wherein in the ligand density gradient(s), the ligand concentration decreases towards the ~~centre~~ center of the support.

Claim 7 (currently amended): ~~A matrix according to any one of the preceding claims,~~ The separation matrix of claim 1, which matrix is a chromatography matrix comprised of a plurality of essentially spherical particles, wherein each particle presents one or more gradient(s) perpendicular to the direction of the liquid flow applied in chromatography.

Claim 8 (currently amended): ~~A matrix according to any one of the preceding claims,~~ The separation matrix of claim 1, wherein at least one gradient is the result of varying pKa values of functional groups of the ligands present on the support.

Claim 9 (currently amended): ~~A matrix according to any one of the preceding claims,~~ The separation matrix of claim 1, wherein at least one gradient is the result of a varying net charge of the ligands present on the support.

Claim 10 (currently amended): ~~A matrix according to any one of the preceding claims,~~ The separation matrix of claim 1, wherein at least one gradient is a continuous and smooth gradient.

Claim 11 (currently amended): ~~A matrix according to any one of the preceding claims,~~ The separation matrix of claim 1, wherein the ligands present on the support provide at least two different functionalities.

Claim 12 (currently amended): ~~A~~ The separation matrix ~~according to~~ of claim 11, wherein said functionalities are selected from the group ~~that consists~~ consisting of

cation exchange ligands, anion exchange ligands, hydrophobic interaction chromatography (HIC) ligands, reversed phase chromatography (RPC) ligands, immobilised metal chelating ligands (IMAC), thiophilic ligands, and affinity ligands.

Claim 13 (currently amended): ~~A matrix according to claim 11 or 12,~~ The separation matrix of claim 11, wherein said at least two different functionalities are present on the same ligand.

Claim 14 (currently amended): ~~A~~ The separation matrix ~~according to~~ of claim 11, wherein the ligands present zwitterionic functionalities.

Claim 15 (currently amended): ~~A matrix according to claim 11 or 12,~~ The separation matrix of claim 11, wherein said at least two different functionalities are present on different ligand kinds, and each such ligand kind provides a separate chemical gradient within the support.

Claim 16 (currently amended): A chromatography column packed with a separation matrix comprised of a porous support; and ligands coupled to the surfaces of ~~a~~ said porous support, wherein the ligands provide at least one chemical gradient within the support.

Claim 17 (currently amended): ~~A~~ The chromatography column ~~according to~~ of claim 16, wherein the support comprises essentially spherical porous particles and the ligands provide at least one chemical gradient between the ~~centre~~ center and the exterior surface of each porous particle.

Claim 18 (currently amended): ~~A~~ The chromatography column ~~according to~~ of claim 16 ~~or 17~~, wherein at least one gradient is a ligand density gradient formed by a changing density of ligands on the support.

Claim 19 (cancelled)

Claim 20 (currently amended): ~~A~~ In a method of providing a separation matrix ~~comprising~~ containing ligands coupled to the surfaces of a porous support, ~~in which method~~ the improvement comprises providing at least one ligand density gradient ~~is provided~~ by solvent-controlled diffusion of at least one reagent into the porous support.

Claim 21 (currently amended): ~~A~~ The method ~~according to~~ of claim 20, wherein the solvent-controlled diffusion is obtained by contacting a first solvent comprising said reagent(s) with the support, in the pores of which a second solvent is present, said first and second solvents presenting different solubilities.

Claim 22 (currently amended): ~~A~~ The method ~~according to~~ of claim 21, wherein the first solvent is aqueous and the second solvent is organic.

Claim 23 (currently amended): ~~A~~ The method ~~according to~~ of claim 21, wherein the first solvent is organic and the second solvent is aqueous.

Claim 24 (currently amended): ~~A method according to any one of claims 20-23,~~ The method of claim 20, wherein the diffusion rate is controlled by adjusting one or more conditions selected from the group consisting of temperature; air flow; solvent properties; and concentration and/or nature of functionalities.

Claim 25 (currently amended): ~~A~~ The method ~~according to~~ of claim 24, wherein the diffusion of reagent(s) is assisted by providing an essentially continuous air flow through the reaction mixture during the reaction.

Claim 26 (currently amended): ~~A method according to any one of claims 20-25,~~ The method of claim 20, wherein a ligand density gradient that decreases towards the ~~centre~~ center of the support is provided by diffusion-controlled addition to the porous support of a reagent that comprises at least one functionality.

Claim 27 (currently amended): ~~A~~ The method ~~according to~~ of claim 26, wherein either the reagent or groups present on the surface of the porous support have been activated before the reaction.

Claim 28 (currently amended): ~~A method according to any one of claims 20-25,~~ The method of claim 20, wherein the support presents activated groups.

Claim 29 (currently amended): ~~A~~ The method ~~according to~~ of claim 28, wherein a ligand density gradient that increases towards the ~~centre~~ center of the support is provided by diffusion-controlled addition to the porous support of a first reagent, which comprises deactivating groups, to deactivate in a controlled fashion some of the

surface groups of the support, and subsequent addition of another reagent, which comprises at least one functionality, to couple said at least one functionality to the surface groups that have not been deactivated.

Claim 30 (currently amended): ~~A method according to any one of claims 20-29,~~ The method of claim 20, wherein the reagent comprises two different functionalities in a predetermined ratio to provide to different ligands in the separation matrix.

Claim 31 (currently amended): ~~A method according to any one of claims 20-30,~~ The method of claim 20, wherein the porous support comprises essentially spherical particles.

Claim 32 (currently amended): A method of preparing a separation matrix that comprises ligands coupled to the surfaces of a porous support, which method comprises the ~~step~~ steps of:

(a) providing activatable groups on the surface of a porous support;

(b) activating said groups with an activation agent;

(c) reacting groups activated according to step b) with a compound which comprises at least one functionality;

wherein control of the reactivity in step (c) results in at least one chemical gradient within the support.

Claim 33 (currently amended): ~~A~~ The method ~~according to~~ of claim 32, wherein the reactivity is controlled by the concentration of the compound that comprises the functionalities in step (c).

Claim 34 (currently amended): ~~A method according to claim 32-33,~~ The method of claim 32, wherein the activatable groups of step (a) are carbon-carbon double bonds.

Claim 35 (currently amended): ~~A method according to any one of claims 32-34, which also comprises to provide~~ The method of claim 32, further comprising providing the activatable groups present at the surface of at least one porous particle in a step preceding step (a).

Claim 36 (currently amended): ~~A~~ The method ~~according to~~ of claim 35, wherein the step preceding step (a) comprises ~~to allylate~~ allylating hydroxyl groups present on the surface of a porous support.

Claim 37 (currently amended): ~~A method according to any one of claims 35-36,~~ The method of claim 35, wherein steps (a)-(c) are replaced by a single step ~~wherein~~ of reacting an activated ligand ~~is reacted~~ with the activatable groups present on the surface of the support.

Claim 38 (currently amended): ~~A method according to any one of claims 32-37,~~ The method of claim 32, wherein the activation agent used in step (b) is a halogen.

Claim 39 (currently amended): ~~A method according to any one of claims 32-38,~~ The method of claim 32, wherein at least one chemical gradient is a ligand density gradient.

Claim 40 (currently amended): ~~A method according to anyone of claims 32-38,~~ The method of claim 32, wherein at least one chemical gradient is a continuous and smooth gradient.

Claim 41 (currently amended): ~~A method according to any one of claims 32-40,~~ The method of claim 32, wherein ~~the~~ at least two different functionalities are provided in step (c).

Claim 42 (currently amended): ~~A~~ The method ~~according to~~ of claim 41, wherein said at least two different functionalities are provided by one compound.

Claim 43 (currently amended): ~~A~~ The matrix ~~according to~~ of claim 41, wherein said at least two different functionalities are provided by different compounds.

Claim 44 (currently amended): ~~A method according to any one of claims 32-43,~~ The method of claim 32, wherein two or more chemical gradients are provided in the support, one of which is a ligand density gradient.

Claim 45 (currently amended): ~~A method according to any one of claims 32-44,~~ The method of claim 32, wherein the support comprises porous particles~~, preferably essentially spherical particles,~~ and at least one gradient extends between the ~~centre~~ center and the exterior surface of each porous particle.

Claim 46 (currently amended): A separation matrix prepared by the method of claim ~~20 according to any one of claims 20-45~~.

Claim 47 (currently amended): A process of liquid chromatography, ~~wherein~~ comprising contacting a liquid ~~comprising~~ including at least one target molecule ~~is contacted~~ with a separation matrix, ~~which~~ said separation matrix comprises ligands coupled to the surfaces of a porous support~~,~~; and adsorbing the target molecule(s) ~~are adsorbed~~ to the matrix, wherein the ligands provide at least one chemical gradient within the support.

Claim 48 (currently amended): ~~A~~ The process ~~according to~~ of claim 47, wherein the liquid is applied in a flow direction which is perpendicular to at least one chemical gradient within the support.

Claim 49 (currently amended): ~~A process according to claim 47 or 48, which further comprises~~ The process of claim 47, further comprising a step of eluting the adsorbed target molecule from the matrix by contacting the matrix with an eluent.

Claim 50 (currently amended): ~~A process according to any one of claims 47-49,~~ The process of claim 47, wherein at least one chemical gradient is a ligand density gradient.

Claim 51 (currently amended): ~~A process according to any one of claims 47-50,~~ The process of claim 47, wherein the support comprises essentially spherical porous particles and at least one chemical gradient extends between the ~~centre~~ center and the exterior surface of each porous particle.

Claim 52 (currently amended): ~~A process according to claim 47-51,~~ The process of claim 49, wherein the functional groups of the ligands are zwitterions and the elution is performed at a pH different from that during the adsorption.

Claim 53 (cancelled)

Claim 54 (new): The method of claim 45, wherein the porous particles are spherical particles.

Claim 55 (new): A separation matrix prepared by the method of claim 32.